Polymorphism and Morphotype Transformations in the Rotifer（*Brachionus calyciflorus*）

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Abstract： The relationship of three *Brachionus calyciflorus* morphotypes（two short-spined，single-spined and spineless ones）was studied in the laboratory. Newly hatched females of each morphotype were cultured individually in an inorganic or *Asplanchna*-conditioned mediums，and the morphotype of their offspring were checked at 200 × magnification. Females of each morphotype，cultured in the inorganic medium，could produce offspring of these three morphotypes in a single generation. Females of all three morphotyes could respond to specific *Asplanchna*-substances when they were cultured in *Asplanchna*-conditioned medium. Moreover，we observed that these three morphotype females could coexist in the medium and had three types of egg-bearing females. Based on the above observations we suggest that *B. calyciflorus* was a morphotype complex and has three basic morphotypes.

Key words： *Brachionus calyciflorus*；Morphotype；Polymorphism；Posterolateral spine

萼花臂尾轮虫多态性及形态型转化

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摘要： 在实验室条件下研究了萼花臂尾轮虫 3 种形态型(双短侧棘刺、单短侧棘刺和无侧棘刺)个体之间的关系。从轮虫的培养水体中随机挑出若干 3 种形态型雌体的幼轮虫(龄长 < 24 h)，分别培养在正常培养液和晶囊轮虫培养液过滤液中。将实验轮虫的后代(F1 代)置于 200 倍显微镜下检查其个体的形态型。实验结果表明，在正常培养液中，每种形态型轮虫个体都可以产生 3 种形态型的后代。当培养在晶囊轮虫培养液过滤液中时，这 3 种形态型轮虫的后代都具有 2 个显著增长的侧棘刺。此外，这 3 种形态型个体的轮虫能够共存于同一培养环境中，而且每种形态型个体的轮虫都具有 3 种生殖类型的雌体。由此推测萼花臂尾轮虫是一个由 3 种基础形态型个体组成的形态型轮虫复合体。

关键词： 萼花臂尾轮虫；形态型；多态性；侧棘刺

Morphological polymorphism and cyclomorphosis of planktonic rotifer（*Brachionus calyciflorus*）are mainly induced by fluctuation of environmental factors and chemical signals（kairomones）released by predators（Nayar，1965；Gilbert，1999）. Both field studies and laboratory experiments verify findings that the development of long posterolateral spines in *B. calyciflorus* are induced and are very effective in reducing the capture and ingestion by the predator Asplanchna（Gilbert，1966，1980；Gilbert & Waage，1967）.

According to Gilbert and Waage（1967），the production of the posterolateral spine in *B. calyciflorus* is

associated with a chemical substance secreted by the predatory rotifer *Asplanchna*, and the continuous diluting and abrupt disappearance of *Asplanchna*-substances in field water bodies is often accompanied by a reduction or absence of posterolateral spines. Contrary to this conclusion, Stemberger (1990) reported that even without the *Asplanchna* factor, *B. calyciflorus* could still evolve two short posterolateral spines, and the length of the posterolateral spines was mediated by food concentration. With respect to potential hydrodynamic benefits, whereas development of posterolateral spines may increase drag and reduce sinking rates, they also reduce motility, and therefore increase relative vulnerability of individuals to predators. This paradox makes the evolutionary mechanism of short posterolateral spines in this rotifer species unclear and questionable.

In natural water bodies several morphotypes of *B. calyciflorus* have been reported (Koste, 1978; Pontin, 1978; Kutikova & Fernando, 1995). Among them unspined (UNS), single short-spined (SSS), two short-spined (TSS) and *Asplanchna* induced two long-spined (TLS) morphotypes are common. The present work investigates the relationship among the TSS, SSS and UNS morphotypes.

1　Materials and Methods

A clone of *Brachionus calyciflorus* was collected from an artificial pond (39° 57′ N; 116° 21′ E) and reared successively in an inorganic medium (IOM) (see Gilbert, 1963). The culture medium was renewed every three days and rotifers were fed daily with green algae, *Chlorella pyrenoidosa*, at a density of 4×10^6 cells/mL. The *C. pyrenoidosa* were cultured in modified SE medium. All rotifer and algae cultures and experimental incubations were kept at 20 ± 1.0℃ at a 14∶10 (L∶D) photoperiod [illuminance ≈ 50 Ein/ $(m^2 \cdot s)$] in a diurnal growth chamber.

After culturing the rotifer for a month in the laboratory, three rotifer morphotypes (UNS, SSS and TSS) were found in the medium. Subsequently all newly hatched individuals (old < 24 h) were picked randomly from the culture medium under a dissecting microscope and checked for morphotype under a 200 × microscope. Several individuals of the three morphotypes were piped individually into 96 well tissue culturing plates, containing 0.3 mL culture medium with 4×10^6 cells/mL *C. pyrenoidosa*. Everyday, surviving females together with their attached eggs were transferred to fresh medium, and the morphotypes of their offspring (F1 generation) of each morphotype were checked at

200 × magnifications. The experimental design consisted of three replicates varying in mother numbers with 24 to 25 of UNS, 6 of SSS and 20 to 24 of TSS. Experiments were terminated after three days.

Morphotypes of the F1 generation was recorded when the culture medium was replaced by *Asplanchna*-conditioned medium (ACM). ACM was prepared by collecting filtrate from *A. brightwelli* culture medium (Gilbert, 1966). *Asplanchna brightwelli* was isolated from an intermittent pond (39°56′N; 116°22′E), cultured in the IOM, and fed with *B. calyciflorus*. Everyday, ACM was refreshed and the offspring were examined as previously described. Furthermore, if the F1 generation were two long-spined (TLS), several virgin individuals (old < 24 h) of TLS morphotype were selected and cultured in the IOM, and the morphotype of their F1 generation offspring were checked as previously described. The experimental designs were run in triplicate varying in mother numbers with 3 in UNS, 2 to 3 in SSS, 5 to 6 in TSS and 6 to 8 in TLS. The experiments were continued for three days.

2　Results

Despite our culture of *Brachionus calyciflorus* being devoid of predators, spineless individuals, derived from an intermittent freshwater pond, were capable of developing two short posterolateral spines, and sometimes a single posterolateral spine (Fig. 1, A1, B1, C1).

In the IOM, offspring of UNS, SSS and TSS females expressed morphotype polymorphism. Maternal females of each morphotype could produce individuals of all three morphs. The morphotype of mother females had no significant influence on the proportions of different morphological offspring in the F1 generation (One-way ANOVA, $n = 3$, $P > 0.05$). The majority of individuals in the F1 generation showed the same morph as their mothers, except for the SSS females, of which the SSS offspring in the F1 generation were less than the UNS and TSS morphologies (Tab. 1).

When cultured in the ACM, almost all daughters of females of each morphotype were of the TLS morphotype (posterolateral spine length to body length ratios: 0.55 ± 0.07, $n = 20$) (Tab. 2), which were not affected by the morphotype of the mother females (One-way ANOVA, $n = 3$, $P > 0.05$). After being returned to IOM, most offspring of TLS females were TSS morphotype with few individuals remaining within the TLS morphotype.
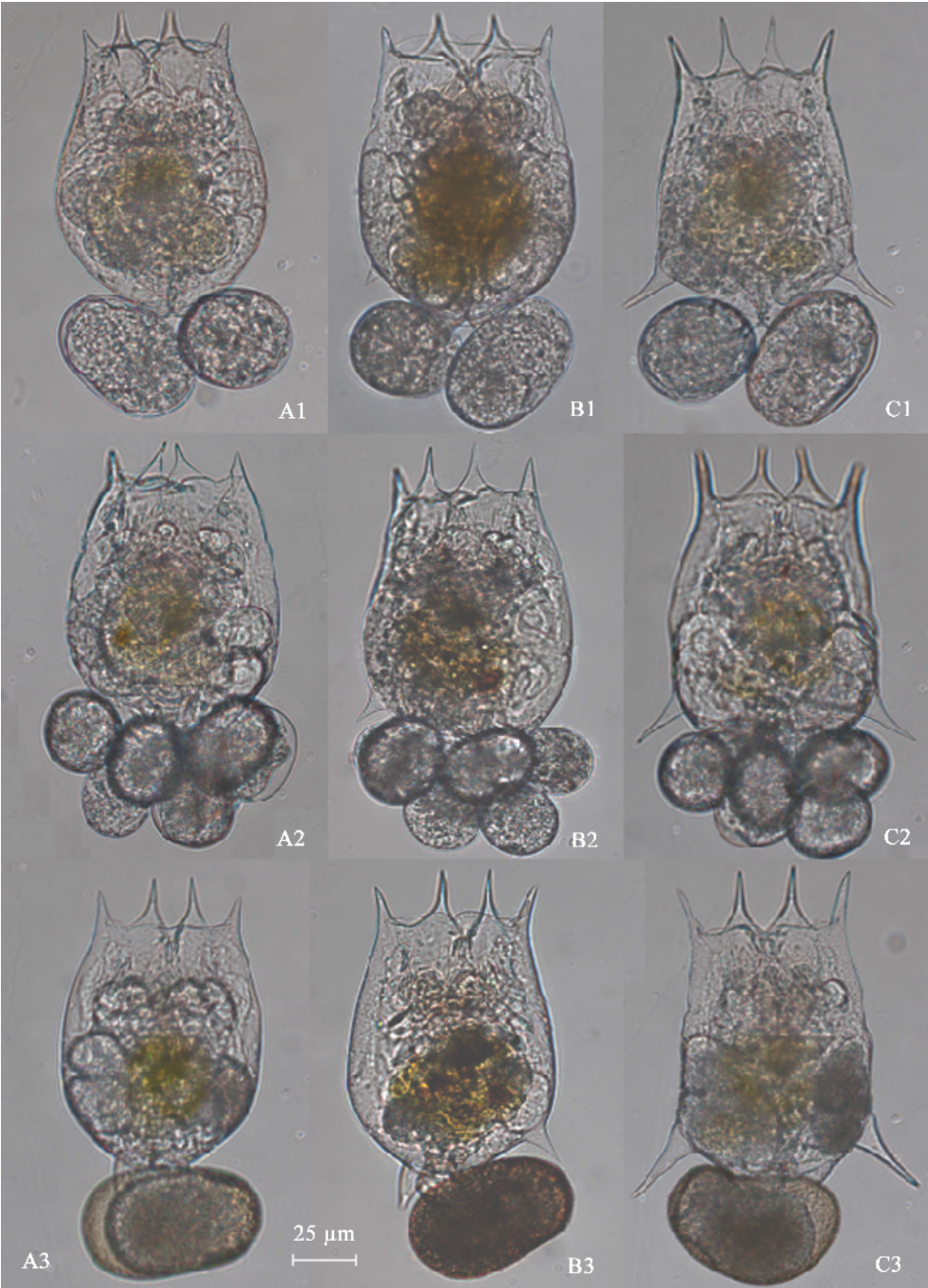
Fig. 1　Three types of egg-bearing females of three morphotypes of *B. calyciflorus*

A1, A2, A3: amictic, male and resting egg-bearing female of UNS; B1, B2, B3: amictic, male and resting eggs bearing female of SSS; C1, C2, C3: amictic, male and resting eggs bearing female of TSS.

Tab. 1　Percentage of different morphological offspring in F1 generation of UNS, SSS, and TSS mothers

Maternal morphotypes	Total observed number	UNS(%)	SSS(%)	TSS(%)
UNS(74)	378	58.5 ± 20.3	12.7 ± 4.5	28.9 ± 17.2
SSS(18)	86	51.0 ± 17.3	13.8 ± 9.7	32.1 ± 8.0
TSS(64)	295	27.5 ± 14.2	11.6 ± 1.5	54.2 ± 15.3

Total numbers of maternal females in experiments are shown in parenthesis.

The mean ± standard error values based on three replicate recordings.

Tab. 2 Percentage of different morphological offspring in F1 generation of four morphotypes mothers in the ACM and IOM

Maternal morphotypes	Type of medium	Total observed number	UNS(%)	SSS(%)	TSS(%)	TLS(%)
UNS(9)	ACM	37	–	–	2.1 ± 2.19	7.9 ± 2.1
SSS(8)		24	–	–	–	100.0 ± 0.0
TSS(16)		27	–	–	3.1 ± 3.1	96.9 ± 3.1
TLS(20)	IOM	40	–	–	91.2 ± 7.7	8.8 ± 7.7

Total numbers of maternal females in experiments are shown in parenthesis.

The mean ± standard error values based on three replicate recordings.

3 Discussion

Our study shows that UNS, SSS and TSS *Brachionus calyciflorus* can coexist in laboratory culture. This simultaneous coexistence of polymorphic morphotypes was also found in the rotifer *Keratella*, in both field and laboratory studies (Gilbert & Stemberger, 1984; Stemberger & Gilbert, 1987; Green, 2005). The magnitude of morphological variation was ascribed to developmental instability (Bradshaw, 1965), and Bradshaw explained phenotypic plasticity with the hypothesis that a genotype would produce variable phenotypes when allowed to develop in different environments. In our experiments, culture environments were suitable for survival and proliferation and were devoid of any predators. Phenotype transformations were achieved within a single generation, not successive generations. Moreover, *B. calyciflorus* were prone to morphotypes transformation in a stable environment, such shifts in morphology explainable through 'developmental conversions' (Smith-Gill, 1983).

Gilbert (1966) reported that newborn long-spined females, induced by culturing in ACM, immediately placed into IOM would hatch spineless individuals from their amictic eggs. Differing with these observations, TLS females produced TSS daughters in our experiments (Tab. 2). Two possible reasons may account for this. Firstly, in this study we used *A. brightwelli* to prepare conditioned medium, which differed with the *A. sieboldi* used by Gilbert (1966). The response of prey to predator can show specificity and may vary with the nature of the stimulus secreted by the predator within the environment (reviewed by Harvell, 1990). Although there is a close species relationship among different predators, obvious response differences in prey may still be found (Stemberger & Gilbert, 1987). Secondly, because of the long-term allopatric evolution of geographical rotifer strains used in the two experiments, the two species might have evolved discriminating or specific predator-response systems.

Brachionus calyciflorus females of each morphotype can produce offspring of these three morphotypes within a single generation, and individuals of these three morphotypes coexist both in the culture medium (this study) and in natural freshwater ponds (personal observations). Moreover, all three morphotyes have three types of egg-bearing females (Fig. 1) and can respond to specific *Asplanchna*-substances (Tab. 2). Finally, *B. calyciflorus* females hatching from resting eggs had these three morphotyes (Unpublished data). Therefore, we suggest that *B. calyciflorus* is a morphotype complex that is composed of three basic morphotypes. The relationship of the three morphotypes is shown in Fig. 2. Aside from Kutikova & Fernando (1995), there is no other field and laboratory data of *B. calyciflorus* to support our suggestion.

In the rotifer *B. quadridentatus*, females hatching from dried sediment had two morphotypes: no posterior spine and short posterior spines; and some clones of females with no posterior spine can produce offspring with short posterior spines in culture medium (Gilbert, 2001). Furthermore, spined individuals have been found in the culture of unspined *B. calyciflorus* after several generations (Stemberger, 1990; see Gilbert, 2001), and spineless, single posterior spine and two asymmetric posterior spine *K. slacki* females emerged in common culture vessels (Gilbert & Stemberger, 1984). Therefore, we predict that in predator-induced polymorphic rotifer species, multi-morphotypes coexist and polymorphic morphology transformations are common phenomena. Further experiments should be designed to verify the phenomena.

Due to the lower proportion in populations operating successive parthenogenetic reproduction (Tab. 1), SSS *B. calyciflorus* was not often observed. We can not explain the evolutionary mechanism of the SSS individuals. Firstly, compared with the UNS females, SSS females cannot reduce their vulnerability to predators. Secondly, the development of single posterolateral spine is costly, because SSS individuals will consume more energy to balance in water than UNS or TSS individuals. The only contribution of SSS individuals in our studies is that they could produce more UNS females.
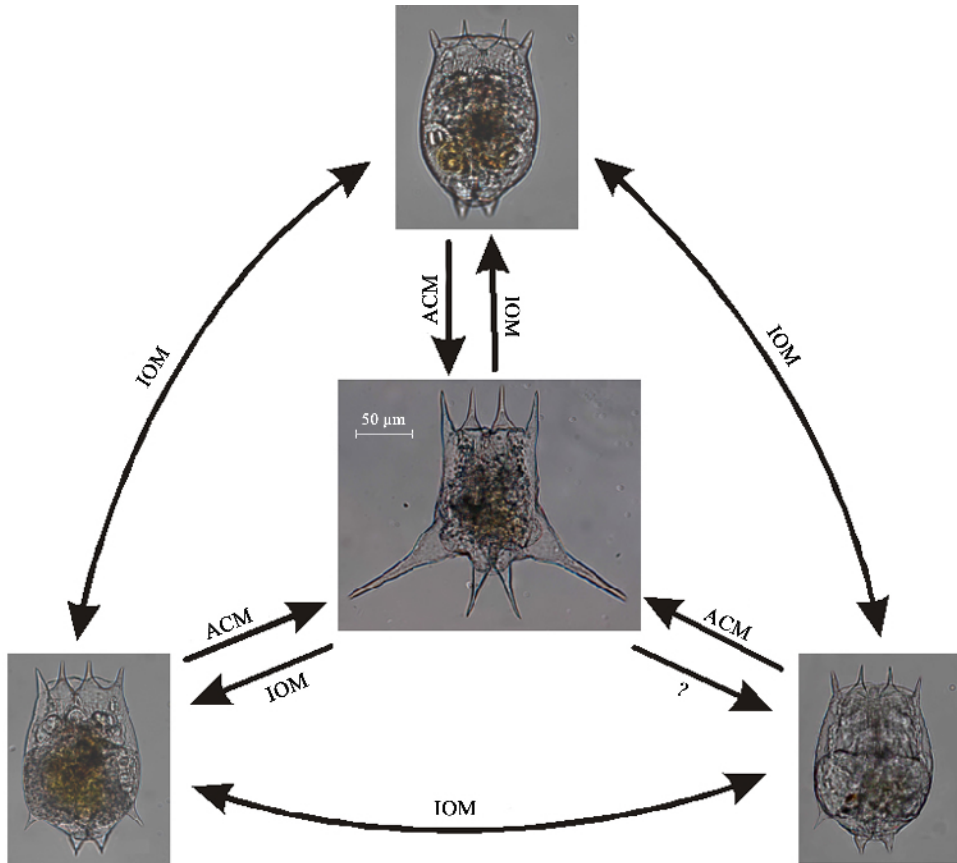
Fig. 2　Relationship of morphotype transformations of *B*. *calyciflorus* in IOM and ACM

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